

Two new *Taifanglania* species identified through DELTA-assisted phenetic analysis

YANFENG HAN, JIANDONG LIANG, ZONGQI LIANG *,
XIAO ZOU & XUAN DONG

swallow112886@yahoo.com.cn jdliang214@yahoo.com.cn zqliang472@yahoo.com.cn
coprinus@126.com juliette9805036@yahoo.com.cn
Institute of Fungus Resources, College of Life Sciences, Guizhou University
Guiyang, China 550025

Abstract — Two new species isolated from soil samples from Mianyang City, Sichuan Province and Nantong City, Jiangsu Province, China, were revealed through classical morphology and DELTA-assisted analyses. Both species are described and illustrated and diagnostic characters revealed through DELTA are discussed. *Taifanglania berberidis* is characterized by brown colony, black reverse, phialides $7.2\text{--}13.5 \times 1.8\text{--}4.2\text{ }\mu\text{m}$, with an ellipsoidal swollen basal portion and fusiform conidia, $4.8\text{--}9.0 \times 1.8\text{--}3.0\text{ }\mu\text{m}$; *T. jiangsuensis* is distinguished by yellow colony, rough or smooth-walled hyphae, phialides $6\text{--}15 \times 1.8\text{--}3.0\text{ }\mu\text{m}$, with a cylindrical swollen basal portion and ellipsoidal or fusiform conidia, $3.6\text{--}6 \times 2.4\text{--}3.0\text{ }\mu\text{m}$, forming chains and often capitate at the top.

Key words — thermotolerant fungi, morphological character, numerical classification

Introduction

The genus *Taifanglania* was established by Liang et al. (2009), who selected *T. hechuanensis* as the type species and accepted nine species in the genus worldwide. *Taifanglania* species are thermophilic fungi that play an important role in cellulose degradation of compost, garbage, and straw (Liang et al. 2007; Kluczek-Turpeinen et al. 2003, 2007; Kluczek-Turpeinen 2007). In addition, they can produce novel active substances (Hill & Pitt 1999) and useful thermophilic enzymes such as laccases (Liang et al. 2007, 2009; Yang et al. 2006).

The recent use of molecular data in systematic analyses has enabled the identification of many new fungi (Luangsa-ard et al. 2004, 2005; Rehner & Buckley 2005; Sung et al. 2007). However, as noted by Hawksworth (2004), only about 11.5% of the known species were represented among the fungal sequences present in Genbank in 2004 and of those, regrettably approximately one-fifth was incorrectly identified. In addition, Paterson (2007, 2008) has

*Corresponding author

reported that mycotoxins present in some cultures may adversely affect DNA sequence analysis. For these reasons, many researchers use polyphasic taxonomy based on molecular, morphological, numerical approaches and physiological data (Houbraken et al. 2007; Samson et al. 2007; Varga et al. 2007a,b). In this regard, the DELTA expert morphology-based system offers many advantages to the classical taxonomic research of animal, plant and microbes that permit digital standardization of morphological characters to make them suitable for taxonomic identification (Carney 2003; Chang et al. 2000; Chen & Chen 2008; Li et al. 1993; Li 1996) and international communication (Chen & Kuoh 2000a,b; Han et al. 2009). In this paper we report and illustrate two new *Taifanglania* species, *T. berberidis* and *T. jiangsuensis*, that were identified using classical morphology in the DELTA system.

Materials and methods

MATERIALS—The eleven *Taifanglania* species used in the study are listed in TABLE 1.

SAMPLE COLLECTION AND STRAIN ISOLATION — Strain GZUIFR-SGQH346 and GZUIFR-HC48.1 were isolated from soil samples of Mianyang City, Sichuan Province and Nantong City, Jiangsu Province, China, respectively. Two grams of soil were added to a flask containing 20 ml sterilized water and glass beads. Each soil suspension was shaken for about 10 min. and then diluted to concentrations of 10^{-1} – 10^{-2} . One ml suspension (10^{-2}) was mixed with Martin medium in a sterilized Petri dish of 9 cm diam. Cultures were incubated at 40°C for 14 days.

STRAIN IDENTIFICATION — The strains were transferred to Czapek agar. After incubation at 40°C for 7 days, the strains were identified based on colony characters and conidiogenous structures according to Liang et al. (2009).

TABLE 1. List of *Taifanglania* spp. for constructing DELTA database

No.	NAMES	REFERENCES
1	<i>T. ampullaris</i> (Matsush.) Z.Q. Liang et al.	Matsushima 1971, Liang 2009
2	<i>T. ampulliphora</i> (Matsush.) Z.Q. Liang et al.	Matsushima 1975, Liang et al. 2009
3	<i>T. bififormis</i> (Z.Q. Liang et al.) Z.Q. Liang et al.	Liang et al. 2007, 2009
4	<i>T. cinerea</i> (Z.Q. Liang et al.) Z.Q. Liang et al.	Liang et al. 2006, 2009
5	<i>T. curticateinata</i> (Z.Q. Liang & Y.F. Han) Z.Q. Liang et al.	Han et al. 2007, Liang et al. 2009
6	<i>T. furcata</i> (Z.Q. Liang et al.) Z.Q. Liang et al.	Liang et al. 2006, 2009
7	<i>T. hechuanensis</i> Z.Q. Liang et al.	Liang et al. 2002, 2009
8	<i>T. inflata</i> (Burnside) Z.Q. Liang et al.	Samson 1974, Liang et al. 2009
9	<i>T. major</i> (Z.Q. Liang et al.) Z.Q. Liang et al.	Chu et al. 2004, Liang et al. 2009
10	<i>T. berberidis</i>	(this work)
11	<i>T. jiangsuensis</i>	(this work)

TABLE 2. Characters and character states in *Taifanglania*

#1. Note/	#10. Phialides length/ μm /
#2. Colony/	#11. Phialides width/ μm /
#3. Colony color/ 1. Pale yellow/ 2. Pale-brown/ 3. Gray/ 4. White/ 5. Yellow/	#12. Phialides <shape>/ 1. cylindric/ 2. ovoid/ 3. ellipsoid/ 4. subglobose/ 5. fusiform/
#4. Reverse color/ 1. Yellow/ 2. Dark/ 3. Offwhite/	#13. Conidia surface/ 1. tiny rough/ 2. smooth/
#5. Colony texture/ 1. Compact velvety/ 2. Velvety, powdery or floccose to funiculose/ 3. Short floccose/ 4. Loose velvety/	#14. Conidia <shape>/ 1. ellipsoid/ 2. subglobose/ 3. cylindric/ 4. ovate/ 5. obovoid/ 6. fusiform/ 7. Lemon-shaped/
#6. Vegetative hyphae <width μm >/	#15. Conidia width/ μm /
#7. Hyphae surface/ 1. tiny rough/ 2. smooth/	#16. Conidia length/ μm /
#8. Conidiophore/ 1. present (simple) 2. lacking/	#17. Conidial chain/ 1. long/ 2. short/
#9. Phialides/	#18. Inhabit/ soil/

THE PHENETIC TREE GENERATED BY DELTA SYSTEM —Diagnostic characters and character states from the selected *Taifanglania* strains were entered into DELTA system as described by Han et al. (2009) to form the database (TABLE 2). According to the program CONFOR with the “todis” directives of Editor, the phenetic tree was generated by DELTA system using the PCLASS program.

New species

Taifanglania berberidis Y.F. Han & Z.Q. Liang, sp. nov.

FIG. 1

MYCOBANK MB 516503

In agar *Czapekii*, coloniae 75–80 mm diam., 14 diebus ad 40°C, planae, brunnea. Conidiophora absentia. Phialides singulares, 7.2–13.5 $\times$ 1.8–4.2 μm , e basi inflata ellipsoidea in collum distinctum apice inspissato angustatae. Conidia fusiformia, 4.8–9.0 $\times$ 1.8–3.0 μm , catenata, interdum et capitata ad extremam.

Holotypus GZUIFR-SGQH346 isolatus, *e soli*, Mianyang City, Provincia Sichuan, China. VIII, 2006, Y.F.HAN, in Guizhou Univ, conservatur.

ETYMOLOGY: *berberidis* (Latin), referring to the associated plant genus.

COLONY on Czapek agar reaching 75–80 mm diam. within 14 days at 40°C, flat, felty, with brown center and gray margin, irregularly radially grooved, faintly wavy at the margin. Reverse black. VEGETATIVE HYPHAE hyaline, smooth-walled, 1.2–3.0 μm in diam. PHIALIDES single, borne directly on the vegetative hyphae, 7.2–13.5 $\times$ 1.8–4.2 μm , with an ellipsoidal swollen basal portion, tapering into a distinct neck. CONIDIA hyaline, smooth-walled, fusiform, 4.8–9.0 $\times$ 1.8–3.0 μm , forming chains and sometimes capitate at the top.

DISTRIBUTION: Sichuan Province, China.

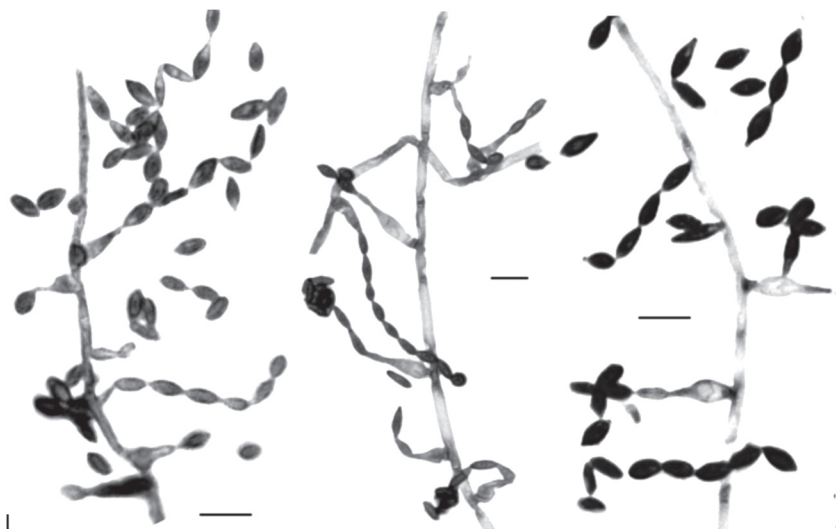


FIG. 1. Conidiogenous structures of *Taifanglania berberidis*. Bars = 10 μm

COMMENTS: Other *Taifanglania* species characterized by fusiform conidia are *T. inflata*, *T. jiangsuensis*, *T. hechuanensis*, and *T. biformis*. The following characters separate those species from *T. berberidis*: *T. inflata* is mesophilic, *T. jiangsuensis* colonies are yellow, the reverse of *T. hechuanensis* colonies is gray, and *T. biformis* has an echinate conidiophore. *Taifanglania berberidis* can be distinguished by its thermophilic character, brown colony with the black reverse, single phialides, and often capitate top of conidial chain.

***Taifanglania jiangsuensis* Y.F. Han & Z.Q. Liang, sp. nov.**

FIG. 2

MYCOBANK MB 516504

In agar *Czapekii*, coloniae 45–50 mm diam., 14 diebus ad 40°C, villiformis, flava. Conidiophora absentia. Phialides singulares, 6–15 $\times$ 1.8–3.0 μm , e basi inflata cylindrica in collum distinctum apice inspissato angustatae. Conidia ellipsoidea vel fusiformia, 3.6–6 $\times$ 2.4–3.0 μm , catenata, saepea et capitata ad extremam.

Holotypus GZUIFR-HC48.1 *isolatus, e soli*, Nantong City, Provincia Jiangsu, China. IV, 2005, Y.F.HAN, in Guizhou Univ, conservatur.

ETYMOLOGY: *jiangsuensis* (Latin), referring to Jiangsu Province, where the type locality is situated.

COLONY on Czapek agar reaching 45–50 mm diam. within 14 days at 40°C, villiform, light yellow. Reverse yellow. VEGETATIVE HYPHAE hyaline, rough or smooth-walled, 0.6–1.2 μm diam. PHIALIDES single, sometimes proliferating, borne directly on the vegetative hyphae, 6–15 $\times$ 1.8–3.0 μm , with a cylindrical swollen basal portion, tapering into a distinct neck. CONIDIA hyaline, smooth-walled, ellipsoidal or fusiform, 3.6–6 $\times$ 2.4–3.0 μm , forming chains, often capitate at the top.

DISTRIBUTION: Jiangsu Province, China.

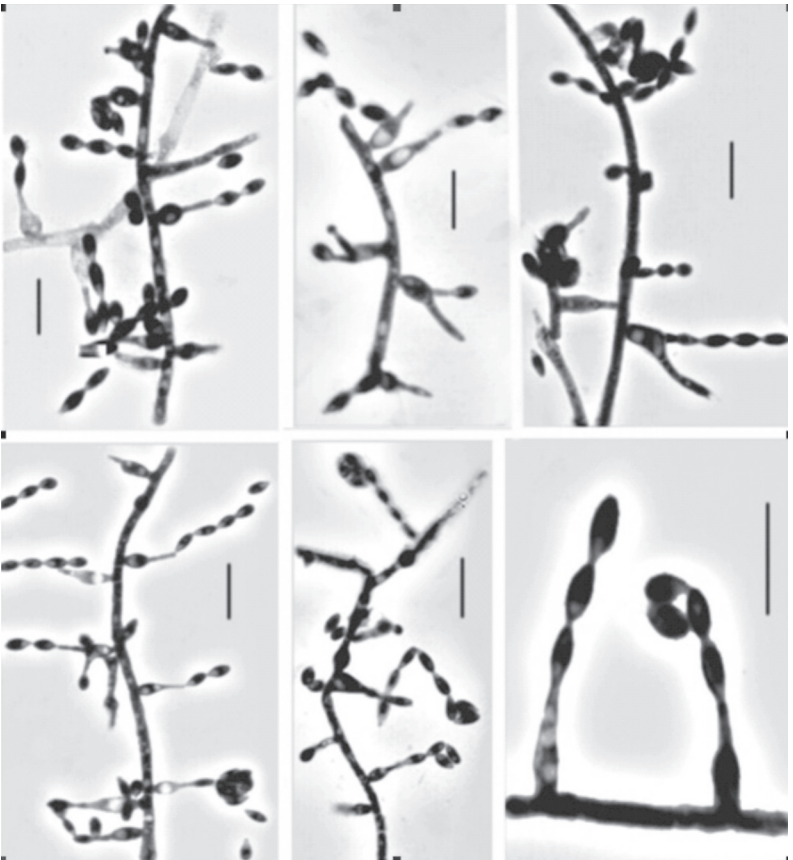


FIG. 2. Conidiogenous structures of *Taifanglania jiangsuensis*. Bars = 10 μm

COMMENTS: Other *Taifanglania* species that produce light colonies on Czapek agar are *T. inflata* and *T. ampullaris*. *T. jiangsuensis*. The fact that *T. inflata* is mesophilic and *T. ampullaris* produces smaller conidia ($2.2\text{--}3.4 \times 2\text{--}2.6 \mu\text{m}$) differentiate those species from *T. jiangsuensis*. Additionally *T. jiangsuensis* may possess either smooth or rough hyphae.

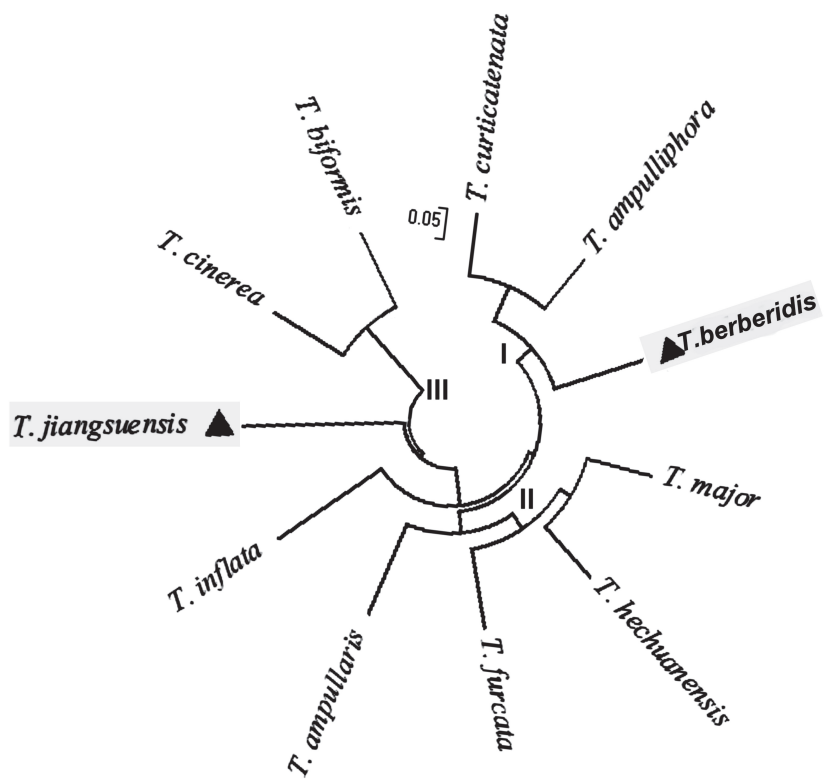


FIG. 3. Phenetic tree generated for *Taifanglania* spp. by DELTA.

Numerical taxonomy

Numerical character classification was not granted special priority, so that all characters selected were weighted equally in this study. The phenetic tree based on the morphological characteristics is presented in FIGURE 3, which separates *T. jiangsuensis* and *T. inflata* from each other and from all other *Taifanglania* species. The remaining species fall into three distinct groups (I–III).

Group I species (i.e., *T. curticatenata*, *T. ampulliphora*, *T. berberidis*) all produce brown colonies on Czapek agar. Both *T. curticatenata* and *T.*

ampulliphora are characterized by rough conidia (Han et al. 2007, Matushima 1975), separating them from *T. berberidis*, which produces smooth, fusiform conidia, which supports *T. berberidis* as an independent species.

Group II species (i.e., *T. major*, *T. hechuanensis*, *T. furcata*, *T. ampullaris*) share light colony color and phialides with ellipsoidal basal portions.

Group III includes *T. cinerea* and *T. biformis*, whose common characters are the villiform colony and conidia that are fusiform, ellipsoidal to cylindrical, and longer than 13 µm. Within the group, the unique biform conidiogenous structures separate *T. biformis* from *T. cinerea* (Liang et al. 2006, 2007).

The new species *T. jiangsuensis*, which stands on an independent branch, can be characterized by its yellow villiform colony, yellow reverse, and tiny rough hyphae.

The phenetic tree generated from morphological characters observed in the eleven *Taifanglania* species using the INTKEY program of DELTA system showed that colony color, colony texture, shape of conidia, reverse color, and conidial length are diagnostic for the identification of *Taifanglania* species.

In conclusion, *T. berberidis* and *T. jiangsuensis* are two new distinctive taxa with the support derived from morphology with the phenetic analysis of DELTA system.

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References

- Carney RS. 2003. Preparation of an interactive key for the northern Gulf of Mexico polychaete taxonomy employing the DELTA/INTKEY system. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS, New Orleans La. OCS Study MMS 2003-065: 38.
- Chang ER, Dickinson TA, Jefferies RL. 2000. Seed flora of La Pérouse Bay, Manitoba, Canada: a DELTA database of morphological and ecological characters. Canadian Journal of Botany 78: 481–496.
- Chen CHK, Kuoh CS. 2000a. The application of DELTA database system on taxonomy. Quarterly Journal of Chinese Forestry 33: 573–583.
- Chen CHK, Kuoh CS. 2000b. The genus *Bromus* L. (Poaceae) in Taiwan: A DELTA database for generating key and descriptions. Taiwania 45: 311–322.
- Chen X, Chen X. 2008. Application of new DELTA system in plant taxonomy — Study on *Festuca* L. as an example. Guihaia 28: 759–763.
- Chu HL, Liang ZQ, Han YF. 2004. A thermotolerant *Paecilomyces inflatus* var. *major* Liang Z.Q., Chu H.L., Han Y.F. var. nov. which produces laccase. Journal of Fungal Research 2(3): 43–46.
- Han YF, Liang ZQ, Chu HL. 2007. A new thermophilic species of *Paecilomyces*, *Paecilomyces curticatenuatus*. Mycosystema 26(1): 13–16.

- Han YF, Zhang YW, Liang JD, Liang ZQ. 2009. Studies on the genus *Paecilomyces* in China. Application of DELTA expert system on the entomopathogenic *Paecilomyces* sensu lato. *Mycotaxon* 109: 75–84.
- Hawksworth DL. 2004. Fungal diversity and its implications for genetic resource collections. *Studies in Mycology* 50: 9–17.
- Hill RA, Pitt AR. 1999. Hot off the press. *Natural Product Reports* 16: 3–6.
- Houbraken JM, Varga J, Meijer M, Frisvad JC, Samson RA. 2007. Polyphasic taxonomy of *Aspergillus* section *Usti*. *Studies in Mycology* 59: 107–128.
- Kluczek-Turpeinen B. 2007. Lignocellulose degradation and humus modification by the fungus *Paecilomyces inflatus*. Academic Dissertation in Microbiology, Division of Microbiology Department of Applied Chemistry and Microbiology University of Helsinki. Helsinki 1–84.
- Kluczek-Turpeinen B, Majjala P, Hofrichter M, Hatakka A. 2007. Degradation and enzymatic activities of three *Paecilomyces inflatus* strains grown on diverse lignocellulosic substrates. *International Biodeterioration & Biodegradation* 59 (4): 283–291.
- Kluczek-Turpeinen B, Tuomela M, Hatakka A, Hofrichter M. 2003. Lignin degradation in a compost environment by the deuteromycete *Paecilomyces inflatus*. *Applied Microbiology and Biotechnology* 61: 374–379.
- Li DC, Zhang TY, Wu JS. 1993. A preliminary study of numerical taxonomy of the genus *Alternaria* (*Hyphomycetes*). *Acta Mycologica Sinica* 12: 232–239.
- Li JJ. 1996. DELTA system-the international standard for plant taxonomy description language. *Act. Phyto. Sin.* 34: 447–452.
- Liang ZQ, Han YF, Chu HL. 2006. Studies on the genus *Paecilomyces* in China. IV. Two new species of *Paecilomyces* with monophialides. *Mycotaxon* 97: 13–20.
- Liang ZQ, Han YF, Chu HL. 2007. A new thermotolerant *Paecilomyces* species which produces laccase and a biform sporogenous structure. *Fungal Diversity* 27: 95–102.
- Liang ZQ, Han YF, Chu HL, Fox RTV. 2009. Studies on the genus *Paecilomyces* in China V. *Taifanglania* gen. nov. for some monophialidic species. *Fungal Diversity* 34: 69–77.
- Luangsa-ard JJ, Hywel-Jones NL, Samson RA. 2004. The polyphyletic nature of *Paecilomyces* sensu lato based on 18S-generated rDNA phylogeny. *Mycologia* 96:773–780.
- Luangsa-ard JJ, Hywel-Jones NL, Manoch L, Samson RA. 2005. On the relationships of *Paecilomyces* sect. *Isarioidea* species. *Mycological Research* 109:581–589.
- Matushima T. 1971. Microfungi of the Solomon Islands and Papua-New Guinea. Published by the Author, Kobe: 42.
- Matushima T. 1975. *Icones Microfungorum a Matushima Lectorum*. Published by the Author, Kobe: 104.
- Paterson RRM. 2007. Internal amplification controls have not been employed in fungal PCR hence potential false negative results. *Journal of Applied Microbiology* 102: 1–10.
- Paterson RRM. 2008. Fungal Enzyme Inhibitors as Pharmaceuticals, Toxins and Scourge of PCR Current Enzyme Inhibition 4: 46–59.
- Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1- α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97(1): 84–98.
- Samson RA. 1974. *Paecilomyces* and some allied hyphomycetes. *Studies in Mycology* 6: 1–119.
- Samson RA, Hong S, Peterson SW, Frisvad JC, Varga J. 2007. Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. *Studies in Mycology* 59: 147–203.
- Sung GH, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B, Spatafora JW. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Studies in Mycology* 57: 5–59.

- Varga J, Due M, Frisvad JC, Samson RA. 2007a. Taxonomic revision of *Aspergillus* section Clavati based on molecular, morphological and physiological data. *Studies in Mycology* 59: 89–106.
- Varga J, Frisvad JC, Samson RA. 2007b. Polyphasic taxonomy of *Aspergillus* section Candidi based on molecular, morphological and physiological data. *Studies in Mycology* 59: 75–88.
- Yang SQ, Yan QJ, Jiang ZQ, Li LT, Tian HM, Wang YZ. 2006. High-level of xylanase production by the thermophilic *Paecilomyces thermophila* J18 on wheat straw in solid-state fermentation. Bioresource Technology 97: 1794–1800.